

CONDENSED TANNINS: A NOVEL REARRANGEMENT OF PROCYANIDINS AND PRODELPHINIDINS IN THIOLYTIC CLEAVAGE

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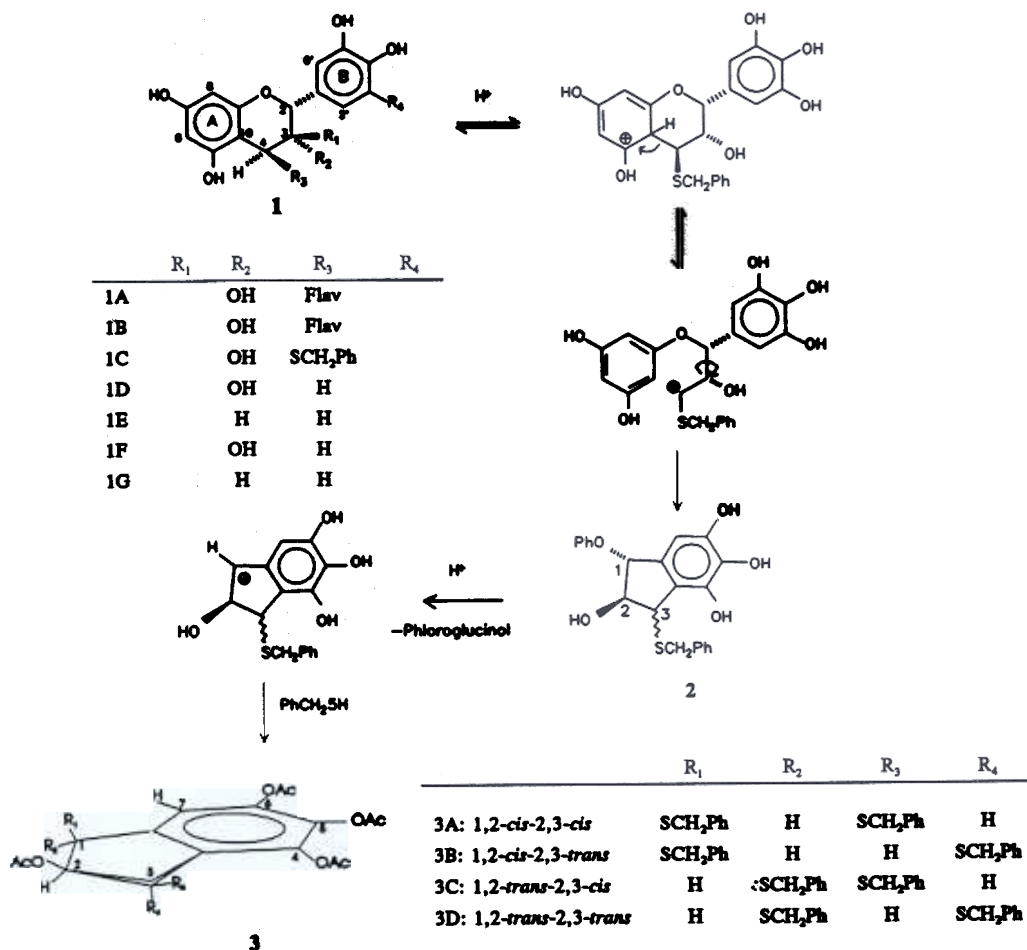
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Abstract—Conditions commonly used for the thiolytic cleavage of interflavanoid bonds of condensed tannins also result in cleavage of the C4 to C10 bond of flavan units. Subsequent electrophilic attack of the C4 carbocation on the C2' or C6' of the B-ring, and loss of phloroglucinol (the A-ring), result in the formation of a mixture of 1,3-dithiobenzyl-2,4,5,6-tetrahydroxy indane diastereomers.

Cleavage of procyanidin (1A, phloroglucinol A-ring, catechol B-ring) and prodelphinidin (1B, phloroglucinol A-ring, pyrogallol B-ring) polymers in the presence of acid with thiols as capture nucleophiles (Scheme 1) is a well-established analytical method, yielding flavan thioethers from the extender units and flavan-3-ols from the terminal unit of the polymers.¹ Gupta and Haslam² reported that, with excess thiol and long reaction times, only monomeric flavan thioethers and the flavan-3-ol terminal unit were recovered and suggested that the reaction could be used to estimate procyanidin molecular weights. In addition, *micro*-thiolytic cleavage reactions have been developed in an attempt to provide quantitative analyses of the proportions of various flavan units in "mixed" proanthocyanidin polymers.^{3,4}

However, in our hands, application of thiolytic cleavage to study of the condensed tannins from tree barks and nut shells has not provided quantitative yields of monomeric flavan cleavage products.⁵ During the thiolysis of condensed tannins from pecan nut pith, it was noted that significant amounts of phloroglucinol were routinely produced. A hypothesis to explain the generation of phloroglucinol in these reactions is shown in Scheme 1. Opening of the pyran ring between C-4 and C-10 of 1 is comparable to cleaving the interflavanoid bond under acidic conditions. Electrophilic attack of the C-4 carbocation on C-2' or C-6' of the B-ring gives ring closure to 2 with two diastereomers possible. Cleavage of the ether linkage at C-1 of 2 liberates phloroglucinol (the original A-ring) and allows a nucleophile to attack at that position. In the presence of phenylmethanethiol, the 1,3-dithiobenzyl-2,4,5,6-tetrahydroxy indane derivative 3 would be the expected product and this could possibly occur as a mixture of four isomers, *i.e.* 1,2-*cis*-2,3-*cis* (3A), 1,2-*cis*-2,3-*trans* (3B), 1,2-*trans*-2,3-*cis* (3C), and 1,2-*trans*-2,3-*trans* (3D) (Scheme 1).

Condensed tannins isolated from pecan nut pith are a mixture of oligomeric proanthocyanidins made up of epigallocatechin (1D), gallocatechin (1E), and epicatechin (1F) extender units in the approximate ratios of 5:2:1 with either catechin (1G) or gallocatechin as terminal units.⁶ Small amounts of phloroglucinol were generated under thiolysis conditions (105°C, HOAc, 24 hrs.) from procyanidins (pine tannin, epicatechin-4-thiobenzyl ether, and catechin-(4 α -8)-



Scheme 1.

catechin) as well as from prodelphinidins (pecan nut pith tannins, gallo catechin-(4 α -8)-epigallocatechin). Visual comparison of 2D-TLC plates indicated that the thiolysis of prodelphinidins produced greater amounts of phloroglucinol compared to the procyanidin reactions.

To test the hypothesis proposed in Scheme 1, pecan nut pith extract was separated into low molecular oligomers, a tannin-carbohydrate complex and a "purified" condensed tannin.⁷ After thiolysis of the condensed tannin fraction, the reaction mixture was extracted with ethyl ether and, following evaporation of the ether, the ether-soluble material was acetylated (pyridine/acetic anhydride, 1:1). The acetylated products were chromatographed on a silica column with benzene-acetone (1:1) followed by increasingly higher percentages of acetone in benzene. The early eluting fractions were combined and further purified by preparative TLC (silica gel, benzene/acetone (9:1). The R_f 0.78 band was isolated and further purified by preparative TLC as above.

^{13}C NMR of the major product showed no resonances in the region of 108–110 ppm corresponding to the C-6 and C-8 carbons of the phloroglucinol A-ring. FAB-MS showed $[\text{M}+\text{Li}]^+$ of 601.1549 for a molecular formula of $\text{C}_{31}\text{H}_{30}\text{O}_8\text{S}_2\text{Li}$, consistent for the peracetate derivative of 3. ^1H NMR spectroscopy showed the isolate to be a mixture of at least three isomers in a ratio of 2:1.2:1 (Table 1).

Table 1. Selected ^1H n.m.r. Chemical Shifts (in p.p.m.) and coupling constants (in Hz).^a

Yield Ratio	ArH	H-1	H-2	H-3
2	6.94, d $J_{\text{Ar},1}=1.5$ $J_{\text{Ar},3}=1.5$	4.48, dd $J_{\text{Ar},1}=1.5$ $J_{1,2}=5.0$	5.42, dd $J_{1,2}=5.0$ $J_{2,3}=1.5$	4.01, d $J_{\text{Ar},3}=1.5$ $J_{2,3}=1.5$
1.2	7.03, m	4.13, d $J_{1,2}=7.0$	5.35, dd $J_{1,2}=7.0$ $J_{2,3}=6.8$	4.51, d $J_{2,3}=6.8$
1	6.83, d $J_{\text{Ar},1}=1.3$	4.10, dd $J_{\text{Ar},1}=1.3$ $J_{1,2}=5.5$	5.58, dd $J_{1,2}=5.5$ $J_{2,3}=5.5$	4.14, d $J_{2,3}=5.5$

^aRecorded on a Bruker AM-300 spectrometer in CDCl_3 .

Indane derivatives such as 3 could exist as four possible stereoisomers each of which could exist in two conformations. Therefore, a modified Karplus equation as compiled by PCMODEL software package⁹ was used to predict vicinal coupling constants for different conformers after full geometry optimization by the MMX⁸ molecular mechanics force field (Table 2). Conformational analyses show that the puckering of the cyclopentene ring will be controlled mainly by 1,3-diaxial interactions and allylic 1,3-strain^{9,10} between the thiobenzyl group at C-3 and the acetoxy at C-4. The preferred conformation as predicted by MMX force field for the *cis-trans* diastereomer has the bulky C-3 thiobenzyl

Table 2. Predicted dihedral angles and coupling constants in (Hz).

Compound	C3	Dihedral Angle	Degrees	3JHH
3A	up	$\text{H}_1\text{-C}_1\text{-C}_2\text{-H}_2$	-20	7.23
		$\text{H}_2\text{-C}_2\text{-C}_3\text{-H}_3$	+17	7.54
	down	$\text{H}_1\text{-C}_1\text{-C}_2\text{-H}_2$	+15	8.63
		$\text{H}_2\text{-C}_2\text{-C}_3\text{-H}_3$	-13	8.71
3B	up	$\text{H}_1\text{-C}_1\text{-C}_2\text{-H}_2$	-38	4.73
		$\text{H}_2\text{-C}_2\text{-C}_3\text{-H}_3$	-92	0.74
	down	$\text{H}_1\text{-C}_1\text{-C}_2\text{-H}_2$	+28	7.53
		$\text{H}_2\text{-C}_2\text{-C}_3\text{-H}_3$	-155	8.38
3C	up	$\text{H}_1\text{-C}_1\text{-C}_2\text{-H}_2$	+106	1.29
		$\text{H}_2\text{-C}_2\text{-C}_3\text{-H}_3$	+22	7.01
	down	$\text{H}_1\text{-C}_1\text{-C}_2\text{-H}_2$	+147	7.08
		$\text{H}_2\text{-C}_2\text{-C}_3\text{-H}_3$	-16	8.57
3D	up	$\text{H}_1\text{-C}_1\text{-C}_2\text{-H}_2$	+96	0.77
		$\text{H}_2\text{-C}_2\text{-C}_3\text{-H}_3$	-93	0.74
	down	$\text{H}_1\text{-C}_1\text{-C}_2\text{-H}_2$	+151	7.77
		$\text{H}_2\text{-C}_2\text{-C}_3\text{-H}_3$	-153	7.97

group in a pseudoaxial orientation, presumably to alleviate the allylic 1,3-strain. Such an orientation resulted in C-2 being puckered upwards and gave dihedral angles $H1-C1-C2-H2 = 38^\circ$ and $H2-C2-C3-H3 = 92^\circ$. These suggest vicinal couplings $J_{1,2} = 4.73$ Hz and $J_{2,3} = 0.74$ Hz. Data in Table 2 indicate that such couplings are only possible for the *cis-trans* diastereomer 3B in the mentioned conformation. Of the four possible isomers (Scheme 1), the dominant diastereomer thus corresponded to the 1,2-*cis*-2,3-*trans* product 3B based on the $^3J_{H-H}$ couplings and supported by theoretical calculations. Data for the remaining diastereoisomers are not as definitive (compare $^3J_{H-H}$ in Tables 1 and 2).

The identification of 3B supports the proposed rearrangement and invalidates the use of extended thiolyses to provide meaningful estimates of molecular weights. These results also call to question the use of thiolytic cleavage as a means of obtaining "quantitative" information on the composition of "mixed" proanthocyanidin polymers. It should also be noted that the cleavage of 2 may occur to a lesser extent than of 1 so that the phloroglucinol generated may not be entirely indicative of the total amount of rearrangement.

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